HOPKINSIAXANTHIN, A XANTHOPHYLL OF THE SEA SLUG HOPKINSIA ROSACEA

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Carotenoid pigments of land animals are usually one or more of the common, yellow constituents of their vegetable food (Zechmeister, 1934). By contrast, carotenoid pigments of marine animals, especially invertebrates, are seldom identical with the yellow constituents of marine plants (Fox, 1947; Lederer, 1940).

Another example of a carotenoid pigment found thus far only in a marine animal is the unusual rose-pink coloring matter of the nudibranch mollusk *Hopkinsia rosacea*. Hopkinsia is found in such small numbers along the coast of California that only one or two organisms, weighing but a gram or two, have been available at any one time. As a result, studies of the extracted pigment have been restricted to determinations of optical properties such as the characteristic spectral absorption curves, relative adsorbability in Tswett adsorption columns, phasic behavior and color reactions. These properties provide a basis for some deductions regarding the molecular structure of the Hopkinsia pigment. They indicate that this substance, which has not been described before, is a xanthophyll-like carotenoid. In accordance with widely accepted carotenoid nomenclature, this molluscan pigment is called hopkinsiaxanthin.

Experimental

Hopkinsia rosacea was collected at low tide at Moss Beach, San Mateo County, and at the Monterey Peninsula, Monterey County, California. When removed from its habitat, this organism proved to be exceptionally delicate and fragile; hence, specimens were brought to the laboratory in moist seaweed and placed immediately in about 100 ml. of methanol or ethanol. These alcohols removed all the pigment and yielded orange-yellow solutions. Pigments in these alcoholic extracts were transferred to petroleum ether. To this end, about 50 ml. of petroleum ether (B. P. 50–70°) and a large volume of water or of salt solution were added to the alcoholic solutions. When dissolved in petroleum ether, the extracted pigments formed a yellow solution in contrast to the orange-yellow color of the alcohol solution.

Adsorption of the petroleum ether solution of the extracted pigments on a column of powdered sugar (2.4 by 10 cm.) yielded a red-orange zone near the top of the adsorbent. When the adsorbed pigments were washed with petroleum ether plus 0.25 per cent n-propanol, most of the coloring matter moved rapidly through the column as a red-orange zone which contained the hopkinsiaxanthin. The colorless percolate below this red-orange zone indicated that carotenes and esters of hydroxy carotenoids were absent, because most of these substances are not adsorbed on columns of sugar. Above the red-orange hopkinsiaxanthin in the Tswett column,

there appeared about five pale, indistinct, red-orange and yellow zones which were not examined further.

Hopkinsiaxanthin, contained in the principal red-orange zone in the adsorption column, was recovered by removal of the adsorbent with a spatula followed by elution of the pigment with alcohol. Readsorption of the pigment on columns of powdered sugar, of Celite and of activated magnesia always yielded a single band. These results indicate that a single pigment had been isolated (Strain, 1942, 1948). This same pigment was obtained from organisms collected in 1943, 1946, and 1947.

The physical and chemical properties of hopkinsiaxanthin are similar to those of the carotenoid pigments, particularly the keto carotenoids. As with the ketonic carotenoids, the color of solutions of hopkinsiaxanthin varies with the solvent. At

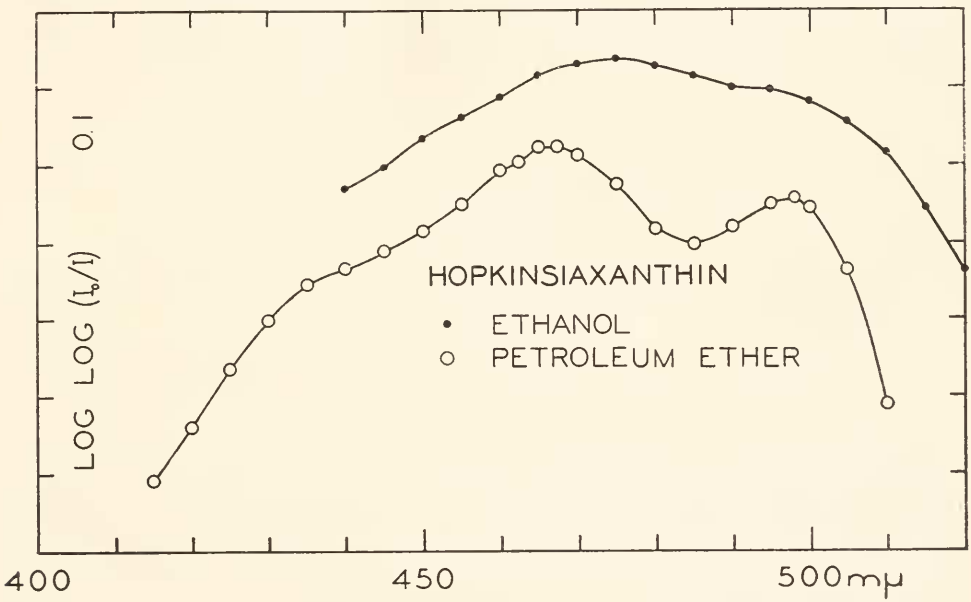

FIGURE 1. Characteristic spectral absorption curves of hopkinsiaxanthin measured in petroleum ether and in 95 per cent ethanol.

equal concentration of pigment, solutions of hopkinsiaxanthin in nonpolar hydrocarbons are a lighter yellow than solutions in polar solvents such as alcohols. This effect, which is readily reversible with change of solvent, is illustrated by the spectral absorption curves reproduced in Figure 1.

As shown by partition experiments, hopkinsiaxanthin is very much more soluble in 90 per cent methanol than in petroleum ether. With 80 per cent methanol, some of the xanthophyll dissolves in the petroleum ether, but most of it remains in the alcohol layer. With 70 per cent methanol, most of the pigment dissolves in the petroleum ether layer.

The adsorbability of hopkinsiaxanthin varies with the adsorbent and the solvent. This xanthophyll is strongly adsorbed on sugar or on Celite when petroleum ether is used as the solvent. It is but weakly adsorbed on these adsorbents when alcohol,

petroleum ether plus alcohol or petroleum ether plus acetone are used as solvents. From these polar solvents the hopkinsiaxanthin is so strongly adsorbed on activated magnesia that acids must be added in order to elute the pigment. On powdered sugar and on Celite, the adsorbed pigment usually appears red-orange, but at low concentration it is salmon colored. Adsorbed on magnesia, the pigment is red-orange even when adsorbed at low concentration.

Adsorbability of hopkinsiaxanthin relative to some common plant carotenoids and chlorophylls also varies with the solvent as is illustrated in Table I. In columns of activated magnesia (plus siliceous filter aid), hopkinsiaxanthin is more tenaciously adsorbed than all the common xanthophylls including the very strongly adsorbed rhodoxanthin. Indeed, it is so strongly adsorbed that it cannot be washed along in the adsorption columns with the strongly polar solvents ethanol and acetone.. In this respect, hopkinsiaxanthin resembles the diketonic carotenoid rhodoxanthin, which is strongly adsorbed on columns of magnesia and but weakly adsorbed on columns of powdered sugar or of Celite (Strain, 1948).

TABLE I

Effect of different solvents upon the adsorbability of hopkinsiaxanthin relative to chlorophylls a and b, lutein and cryptoxanthin in columns of powdered sugar

Petroleum ether	Petroleum ether + 5 per cent acetone	Petroleum ether + 0.25 per cent *n*-propanol
Chlorophyll *b*	Chlorophyll *b*	Chlorophyll *b*
Chlorophyll *a*	Chlorophyll *a*	Hopkinsiaxanthin
Hopkinsiaxanthin	Hopkinsiaxanthin	+
Lutein	Lutein	Lutein
Cryptoxanthin	Cryptoxanthin	Chlorophyll *a*
		Cryptoxanthin

Hopkinsiaxanthin exhibits many color reactions that are characteristic of the carotenoid pigments. A solution of the Hopkinsia xanthophyll in diethyl ether shaken with concentrated hydrochloric acid yields a clear blue color in the acid layer, the blue color remaining unchanged for at least one-half hour. A petroleum ether solution treated with concentrated hydrochloric acid fades rapidly and forms a blue precipitate at the interface between the two liquids. A chloroform solution reacts with antimony trichloride yielding a blue color that also fades rapidly. Strong phosphoric acid (85 per cent) extracts all the hopkinsiaxanthin from petroleum ether and yields a blue color in the acid layer.

Strong alkalies convert hopkinsiaxanthin into a pale yellow pigment. For example, when a solution of this xanthophyll in petroleum ether is shaken with a 10 per cent solution of potassium hydroxide in water, the color changes from deep yellow to light yellow, although most of the pigment remains in the petroleum ether. When this petroleum ether solution is adsorbed on sugar, a weakly adsorbed, yellow band is formed. This yellow band is less adsorbed than the original, unaltered xanthophyll. Solutions of potassium hydroxide in methanol extract the hopkinsiaxanthin from the petroleum ether and the pigment fades rapidly.

Hopkinsiaxanthin dissolved in petroleum ether is but slightly affected by traces of iodine plus dimethylaniline (Strain, 1941). This reaction yields small amounts of pigments that are more strongly adsorbed than the unaltered xanthophyll on columns of powdered sugar.

Discussion

The characteristic color reactions of hopkinsiaxanthin and the effect of polar and nonpolar solvents upon the spectral absorption properties (Fig. 1) suggest that this pigment may be a ketonic carotenoid. The weak adsorbability of hopkinsiaxanthin on columns of powdered sugar and the pronounced adsorbability on columns of magnesia (Table I) also support this conclusion.

As indicated by the wavelengths of the absorption bands, the hopkinsiaxanthin molecule contains about 11 double bonds with at least one of these in the form of a carbonyl group. The stability of the pigment toward solutions of iodine suggests that all these double bonds occur in the more stable, trans, spatial arrangement rather than in labile, *cis* configurations.

Because the adsorbability of hopkinsiaxanthin on columns of powdered sugar approximates that of lutein (dihydroxy alpha-carotene), it is possible that the molecule contains one or two hydroxyl groups. The preferential solubility of hopkinsiaxanthin in methanol relative to petroleum ether suggests that there are probably no esterified hydroxyl groups.

With respect to solubility and color reactions, hopkinsiaxanthin resembles only one of the principal carotenoid pigments isolated from marine plants; namely, fucoxanthin. However, the Hopkinsia pigment is not identical with fucoxanthin as shown by the wavelengths of the absorption bands: 466 and 497 mμ for hopkinsiaxanthin in petroleum ether (Fig. 1) and 449 and 477 mμ for fucoxanthin in petroleum ether (Strain, Manning and Hardin, 1944). It also differs from fucoxanthin with respect to adsorbability, for it is more strongly adsorbed than fucoxanthin on columns of magnesia, and it is less strongly adsorbed than fucoxanthin on columns of powdered sugar.

Summary

The striking, rose-pink color of *Hopkinsia rosacea* is due to the presence of a carotenoid pigment, hopkinsiaxanthin. This xanthophyll, which has not been found in plants or in other animals, occurs in the stable, trans configuration and probably contains a carbonyl group.

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